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Applicant:	Masaru YAMAKOSHI et al.	Conf.:	UNKNOWN
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For:	METHOD OF DETECTING MILD IMPAIRED GLUCOSE TOLERANCE OR INSULIN SECRETORY DEFECT		

LETTER SUBMITTING ENGLISH TRANSLATION OF THE PRIORITY DOCUMENTS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

November 29, 2004

Sir:

Attached hereto is a copy of the English translation of the Priority Document in connection with the above-identified application.

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Respectfully submitted,

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To Mr. OIKAWA Kozo

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[Document Name] Specification

[Title of the Invention] Method of Detecting Mild Impaired Glucose Tolerance or Insulin Secretory Defect

[Claims]

[Claim 1]

A method of detecting mild impaired glucose tolerance or impaired glucose tolerance, characterized in that the method comprises:

quantitatively determining myo-inositol level in a sample; and

evaluating a case where the level shows a characteristic value or more as mild or impaired glucose tolerance.

[Claim 2]

A method of detecting insulin early secretory defect or insulin secretory defect, characterized in that the method comprises:

quantitatively determining myo-inositol level in a sample; and

evaluating a case where the level shows a characteristic value or more as insulin early secretory defect or insulin secretory defect

[Claim 3]

The method according to claim 1 or 2, wherein the quantitative determination of myo-inositol level in the sample is carried out using an enzyme.

[Claim 4]

The method according to claim 3, wherein the enzyme is myo-inositol dehydrogenase.

[Claim 5]

The method according to claim 3 or 4, wherein the quantitative determination of the myo-inositol level using the enzyme is carried out by an enzymatic cycling method.

[Claim 6]

The method according to any one of claims 1 to 5, characterized in that the myo-inositol level is quantitatively determined after elimination of sugars other than myo-inositol in the sample.

[Claim 7]

The method according to claim 6, characterized in that two kinds of hexokinases are simultaneously used for the reaction of eliminating sugars other than myo-inositol in the sample.

[Claim 8]

The quantitative determination method according to claim 7, characterized in that said two kinds of hexokinases are ATP-hexokinase and ADP-hexokinase.

[Claim 9]

The quantitative determination method according to any one of claims 5 to 8, characterized in that thio-NAD which is used as a coenzyme in the reaction of quantitatively determining myo-inositol has a final concentration of 0.1 mM or more.

[Claim 10]

The quantitative determination method according to any one of claims 5 to 8 characterized in that thio-NAD which is used as a coenzyme in the reaction of quantitatively determining myo-inositol has a final concentration of 2 to 10 mM.

[Claim 11]

The method according to any one of claims 1 to 10, wherein the sample is obtained before and after glucose load, or before and after a meal.

[Claim 12]

The method according to claim 11, wherein the sample is urine.

[Claim 13]

The method according to any one of claims 1 to 11, characterized in that the sample is urine and the characteristic value is 0 to 20 „g/mg creatinine when measured as an increasing amount of myo-inositol excreted in the urine after 75g glucose load.

[Claim 14]

The method according to any one of claims 1 to 11, characterized in that the sample is urine and the characteristic value is 8 to 12 „g/mg creatinine when measured as an increasing amount of myo-inositol excreted in the urine after 75g glucose load.

[Claim 15]

The method according to any one of claims 1 to 14, characterized in that glucose level in the sample is quantitatively determined in addition to myo-inositol level in the sample.

[Claim 16]

A composition for a quantitative determination of myo-inositol which comprises at least:

i) thio-NAD;

ii) NADH;

iii) myo-inositol dehydrogenase; and

iv) hexokinases.

[Claim 17]

A composition for a quantitative determination according to claim 16, characterized in that said hexokinases are ATP-hexokinase and an ADP eliminating agent.

[Claim 18]

A composition for a quantitative determination according to claim 16 or 17, characterized in that said composition further comprised a buffer selected from Bicine (N,N-Bis(hydroxyethyl)glycine), Tris (Tris(hydroxymethyl)aminomethane), TEA (Triethanolamine), and Tricine (N-Tris(hydroxymethyl)-methylglycine).

[Claim 19]

A composition for a quantitative determination according to any one of claims 16 to 18, characterized in that thio-NAD has a final concentration of 0.1 mM or more.

[Claim 20]

A composition for a quantitative determination according to any one of claims 16 to 19, characterized in that thio-NAD has a final concentration of 2 to 10 mM.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to a method of examining mild impaired glucose tolerance or insulin early secretory defect using a sample such as urine. In addition, the present invention can be applied to a method for predicting or diagnosing a disease that stems from mild impaired glucose tolerance or insulin early secretory defect, such as diabetes mellitus, arteriosclerosis, or hypertension; a method of determining effects of prevention of, treatment of, or medical advice on those diseases; and a method of evaluating therapeutic agents for treatment of those diseases.

[0002]

[Technical Background]

A final goal of diabetic treatment is to prevent the onset of diabetic complications and to inhibit the development thereof. As demonstrated by clinical tests for achieving this goal, it is important to find any abnormality and start treatment thereof at the earliest possible stage [e.g., *Diabetes Research and Clinical Practice*, **28**, 103 (1995)].

Further, it is considered effective as a more advanced preventive method to find individuals with prediabetes or at prestage of diabetes, or individuals with mild impaired glucose tolerance or insulin early secretory defect, who are not prediabetic at present but are highly likely to develop diabetes or prediabetes in the near future, and give them treatment or advice for exercise and dietary. Clinical tests have been conducted to scientifically demonstrate this [e.g., *Diabetes Care*, **21**, 1720 (1998)]. Therefore, detecting individuals with prediabetes will be important for prevention of diabetes mellitus and also complications thereof. Furthermore, diagnosing individuals with mild impaired glucose tolerance or insulin early

secretory defect, who are not prediabetic at present but are highly likely to develop diabetes or prediabetes in the near future, is considered most important for purpose of preventing diabetes mellitus at an earlier date.

[0003]

An example of the diagnostic method for diabetes mellitus is an oral glucose tolerance test. In a 75 gram oral glucose load test, a group of individuals with the fasting blood glucose level being less than 110 mg/dl and the 2-hour postload blood glucose level being less than 140 mg/dl is defined as normal glucose tolerance. In addition, a group of individuals with the fasting blood glucose level being not less than 110 mg/dl but less than 126 mg/dl and the 2-hour postload blood glucose level being less than 140 mg/dl is defined as impaired fasting glycemia (IFG); and a group of individuals with the fasting blood glucose level being less than 126 mg/dl and the 2-hour postload blood glucose level being not less than 140 mg/dl but less than 200 mg/dl is defined as impaired glucose tolerance (IGT); and both groups IFG + IGT are defined as borderline type. A group of individuals with the fasting blood glucose level being not less than 126 mg/dl or the 2-hour postload blood glucose level being not less than 200 mg/dl is defined as diabetes mellitus.

The guideline of the Japan Diabetes Society teaches that among individuals defined as normal glucose tolerance on the basis of only the fasting blood glucose level and the 2-hour postload blood glucose level, those with the 1-hour postload blood glucose levels being 180mg/dl or more are at higher risk of developing diabetes so that they should be handled as the borderline type.

[0004]

The term "impaired glucose tolerance" or "glucose tolerance failure" refers to the condition of an increase in blood glucose level caused by insufficient uptake of blood glucose into peripheral tissues such as skeletal muscle, liver, and adipocyte after glucose is introduced into the blood through meals. In addition, the term "mild impaired glucose tolerance" refers to the cases wherein the increment is slightly higher than that of healthy individuals.

[0005]

Insulin is a hormone secreted from beta cells of pancreas and acts on skeletal muscle, liver and adipose tissue to lower the blood glucose level. The term "insulin early secretory defect" or "insulin secretory defect" refers to the condition of insufficient insulin secretion to uptake a sufficient amount of blood glucose into peripheral tissues such as skeletal muscle, liver, and adipocyte after glucose is introduced into the blood through meals or the like. According to the guideline of the Japan Diabetes Society, the term "impaired early insulin

secretion " refers to the condition in which the insulinogenic index is less than 0.4. Insulinogenic index is defined as „IRI (30-0)/„PG (30-0) wherein „IRI (30-0) represents the difference between the blood insulin level at 30 min after glucose load and that before glucose load; and „PG (30-0) represents the difference between the blood glucose level at 30 min after glucose load and that before glucose load.

[0006]

Assays of blood glucose levels and insulin levels for those diagnoses are invasive procedures that require blood drawing more than once within a short time, giving the subjects considerable pains. Therefore, there is a need for a simple assay with lower invasiveness, which can solve these disadvantages, preferably a noninvasive assay.

On the other hand, the quantitative determination of myo-inositol in a biological sample has been considered useful for the diagnosis of diabetes mellitus and the following reports have been provided.

(a) In diabetes mellitus, there was an increase in the urinary myo-inositol level [Larner J. et al., *New Eng. J. Med.*, **323**, 373-378 (1990)].

(b) No difference was found between normal glucose tolerance and the borderline type with respect to the urinary myo-inositol level [Susumu Suzuki, *Diabetes Care*, **17(12)**, 1465-1468 (1994)].

(c) The borderline type (IFG, IGT) and diabetes mellitus showed higher urinary myo-inositol level than that of normal glucose tolerance after glucose load (JP 2001-190299 A).

[0007]

The above reports (a) and (b) show the results obtained by determining the urinary myo-inositol levels with GC/MS method. The data are problematic in reproducibility and reliability because they varied among different examiners. On the other hand, in report (c), the results are more precise and reliable than those obtained by GC/MS method because they are obtained by determining the urinary myo-inositol level with a high-sensitive myo-inositol assay reagent using an enzyme. In this way, the detection of the group with prediabetes has become possible.

However, the myo-inositol assay reagent used in report (c) has problems including: (i) an insufficient lower limit of detection because of a narrow measurement range and the need of diluting a sample for determination of various urinary myo-inositol levels; and (ii) insufficient avoidance of effects of coexisting substances in urine, particularly glucose. Therefore, the detection of mild impaired glucose tolerance and insulin early secretory defect, which fall within normal glucose tolerance, has been impossible.

Furthermore, if a subject is judged to be normal glucose tolerance on the basis of only blood level before a 75g oral glucose load and that at 2 hours after the glucose load, such judgment does not reflect the change in blood level from 0 to 2 hours. For example, even individuals (with mild impaired glucose tolerance and insulin early secretory defect) who keep a higher blood glucose level from just after the glucose load, and thus are highly likely to develop diabetes or prediabetes in the near future are practically classified in normal glucose tolerance. Here, the term "mild impaired glucose tolerance" refers to a group which is classified in normal glucose tolerance, but shows a slight decrease in glucose tolerance characterized by, when the loading test is carried out and blood samples are collected four times on fasting and at 30 minutes, 1 hour, and 2 hours after the load, (i) an oxyhyperglycemia, i.e., very high blood glucose levels (180 mg/dL or more) at 30 minutes and 1 hour after the load, (ii) a higher blood glucose level than that of healthy individuals at 2 hours after the load although the blood level is less than 140 mg/dL (e.g. not less than 120 mg/dL), (iii) high „PG (e.g. 530 mg/dL or more). Therefore, it can not be anticipated from the public disclosures (a)-(c) to identify healthy and non-healthy individuals (the individuals who are highly likely to develop diabetes or prediabetes in the near future, for example, those with mild impaired glucose tolerance or those classified as boarder type, IFG, IGT, diabetes mellitus) simply by determining myo-inositol levels.

As mentioned above, the conventional technology teaches no method of detecting mild impaired glucose tolerance and insulin early secretory defect, which keep a higher blood glucose level from just after the glucose load, and thus are highly likely to develop to diabetes or prediabetes in the near future.

[0009]

[Problems to Be Solved by the Invention]

The present invention intends to provide a simple assay method for detecting mild impaired glucose tolerance and/or insulin early secretory defect with good reproducibility.

[0010]

[Means to Solve the Problems]

For achieving this object, the present inventors considered that search for any marker for effectively determining mild impaired glucose tolerance and/or insulin early secretory defect was advantageous. As a result of concentrated efforts, whereas myo-inositol is conventionally considered to be useful for detection of insulin resistance and prediabetes (borderline type and diabetes mellitus), the present inventors unexpectedly found that

myo-inositol is also useful as a marker for effectively detecting mild impaired glucose tolerance or insulin early secretory defect.

[0011]

Blood serum, plasma, or urine collected from the human body, or a homogenized extract of living tissue are used as a sample. Urine is preferable because it can be non-invasively obtained.

[0012]

The present inventors continued to develop a high-sensitive quantitative determination assay of myo-inositol and a composition for the assay to provide a simple and cost-effective quantitative determination assay of myo-inositol with a high degree of accuracy (JP 06-61278). This enzymatic assay, which does not require any preliminary treatment, opened a way to obtain reliable data of myo-inositol for the first time. Such development of the high-sensitive quantitative determination assay and the composition for the quantitative determination allowed the first success of providing a method of the present invention for detecting mild impaired glucose tolerance and/or insulin early secretory defect.

[0013]

Furthermore, after the administration of a given amount of glucose to a subject, urine samples were obtained non-invasively from the subject within a given time period and the myo-inositol levels thereof were determined using the myo-inositol assay reagent as described above. The determination revealed that not only individuals with prediabetes (of borderline type, IFG, IGT) and individuals of diabetes mellitus, but also individuals practically showing mild impaired glucose tolerance or a decrease in early insulin secretion in spite of their normal glucose tolerance have higher levels than the characteristic value predetermined from healthy individuals. Therefore, it has been found that the assay reagent of the present invention enables not only the distinction between normal glucose tolerance and non- normal glucose tolerance with progressed impaired glucose tolerance (borderline type, IFG, IGT, diabetes) but also the simple, highly reproducible and efficient distinction of individuals practically showing mild impaired glucose tolerance or a decrease in early insulin secretion in spite of normal glucose tolerance from healthy individuals.

[0014]

In addition, the concentration of myo-inositol in a sample may be very low and some of myo-inositol dehydrogenases used may react weakly with glucose. Thus, the elimination of glucose may be required in advance. A method for the elimination of glucose includes one utilizing extreme chemical stability of myo-inositol and one by modifying glucose using an

enzyme as a catalyst. The method utilizing the chemical stability includes, for example, one by heating a sample in the presence of 6 N HCl to allow the decomposition of sugars except myo-inositol by the acid and recovering myo-inositol which remains in the decomposed product; and one by treating a sample with a reducing agent such as sodium borohydride to reduce sugars having carbonyl groups or formyl groups such as glucose except myo-inositol whereby modifying the sugars to the compounds unreactive with myo-inositol dehydrogenase, i.e., an enzyme for the quantitative myo-inositol assay. The method by modifying glucose using an enzyme as a catalyst includes one by converting glucose in a sample into gluconic acid with glucose oxidase (EC1,1,3,4) and one by converting glucose in a sample into glucose-6-phosphate with hexokinase (EC2,7,1,1). The modified products by an enzyme are compounds which do not react with myo-inositol dehydrogenase, i.e., an enzyme for the quantitative myo-inositol assay. The inventors of the present invention has found out that it is more preferable to eliminate glucose in advance as mentioned above.

[0015]

Further, the present inventors have found that myo-inositol can be determined more accurately when two kinds of kinases, ATP-hexokinase and ADP-hexokinase, are used simultaneously because the influence of sugars in a sample is reduced. In addition, the present inventors have found that the range of myo-inositol determination can be extended by about 10 times of known methods by adjusting thio-NAD level to a final concentration of 0.1 mM or more, preferably 2 to 10 mM. Thus, the present inventors have completed a higher sensitive assay system.

[0016]

The term "characteristic value" refers to a value determined on the basis of an average of myo-inositol levels in urine samples of healthy subjects selected from those of normal glucose tolerance; standard deviation; and ROC (response operating characteristic) curve. When urine samples are used, the increment in urinary myo-inositol excretion between before the glucose load and at a predetermined time after the glucose load is in the range of 0 to 20 „g/mg creatinine; or 5 to 15„g/mg creatinine; or more preferably 8 to 12 „g/mg creatinine. In addition, the characteristic value may be changed if a large-scale examination is conducted in the future and the determination is conducted for individuals clinically identified as healthy. Furthermore, the characteristic value may also vary depending on the selected populations of race, sex, age, etc.

[0017]

The present invention and preferred embodiments thereof will be described in more detail as

below.

According to the present invention, the detection of mild impaired glucose tolerance and insulin early secretory defect is carried out by determining the amounts of myo-inositol excreted in urine of a subject before the glucose load and at a predetermined time after the glucose load using the reagent of the present invention; and making a comparison of an increasing amount or increasing rate of myo-inositol between before and after the glucose load with the characteristic value defined in advance for healthy individuals.

The increasing amount is calculated as a difference between the myo-inositol content at a predetermined time after the glucose load and before the glucose load, and the increasing rate is calculated as a ratio of the myo-inositol content at a predetermined time after the glucose load to that before the glucose load.

[0018]

For the concentration of myo-inositol, an actually determined value may be used, or a relative value with respect to an appropriate standard index may be used for compensating the dilution of urine with drinking water, etc. Preferably, the index is a urinary creatinine level. The subjects include all individuals in addition to those suspected of being lifestyle-related diseases such as diabetes.

[0019]

Any amounts of glucose loaded and any types of the glucose loading methods may be used. However, preferable is an oral administration of an aqueous 75g glucose solution as used in a typical glucose load test or a meal ingestion.

[0020]

Urine samples may be collected before the glucose load and at any times until after 6 hours from just after the glucose load, preferably at 30 minutes to 3 hours after the glucose load. A urine reservation period before collecting is suitably selected from 30 minutes to 3 hours.

[0021]

When using urine as a sample, which means samples are collected by a non-invasive method, there is no need to select the sampling method, time, and place. For instance, such a sample can be easily prepared by a subject at home, office, school, or the like, and the collected urine sample may be transported directly or in a form of urine-immersed filter paper or other suitable forms, these eliminating the need to be tied to medical institutions or the like. Thus, the present invention provides a prominent method in which, when a filter paper or the like is impregnated with urine, the sample dispatched is extracted by a suitable method and provided to the simple and rapid assay of the present invention and then immediately the results is sent

to the subject.

In particular, urinary myo-inositol levels can be monitored as needed while the subject spends everyday life as usual without glucose load. For example, it is possible to grasp the degree of impaired glucose tolerance or the degree of insulin early secretory defect using the maximum myo-inositol level or the difference between the maximum myo-inositol level and the minimum myo-inositol level in a day. In addition, monitoring urinary myo-inositol levels as needed allows the subject to reconsider the diet contents and control the amount of exercise to prevent diabetes or the progress thereof while leading a regular life.

[0022]

The methods of monitoring urinary myo-inositol levels include any type of methods capable of detecting myo-inositol, for example, a method using a test paper onto which an enzyme that acts on myo-inositol is fixed and a method of electrochemically detecting myo-inositol using an electrode as a sensor onto which an enzyme acting on myo-inositol is fixed.

In the test paper method, for example, hydrogen peroxide is generated by oxidase and reacted with peroxidase to generate active oxygen, and the active oxygen causes the oxidation of chromogen for coloration, the intensity of which may be observed. The chromogen includes, but not limited to, potassium iodide, tetramethylbenzidine, N-(3-sulfopropyl)-3,3',5,5'-sodium tetramethylbenzidine, 4-aminoantipyrine, and O-tolidine.

For detecting by means of the sensor, for example, when oxidase is used, hydrogen peroxide generated may be directly measured using an electrode; or the oxidation-reduction current obtained through an electron carrier such as a ferrocene or a quinone derivative or the quantity of the electric current may be measured. Likewise, when dehydrogenase is used, the reduced coenzyme may be directly measured at an electrode; or the oxidation-reduction current obtained through an electron carrier or the quantity of the electric current may be measured. Examples are shown in "Biosensor and Quantitative Assay of Substrate Using the Same (Application No. JP 09-263492)" and the like.

[0023]

In addition, for example, daily monitoring of urinary myo-inositol levels can be more easily carried out by incorporating the above sensor directly into a toilet stool or the like or into a device attached thereto. Such a device may further have functions of memorizing the measurements and of connecting to a terminal of an information processor. In this way, even when the subject stays in a distant place, a medical practitioner or medical institution can keep in contact with the subject through an electric medium to manage vital data; to give a medical advice; and to examine the degree of impaired glucose tolerance and insulin early secretory

defect, leading to review of the diet contents, control of the amount of exercise, improvement of life style, and the medical treatment.

[0024]

In addition, more precise management can be performed by combining the results of the determination and a doctor's observation. Furthermore, because it is possible to determine the risk to develop diabetes by finding the precondition to diabetes, i.e. prediabetes, and also mild impaired glucose tolerance and insulin early secretory defect, which are not prediabetic at present but are highly likely to change to diabetes or prediabetes in the near future, whereas a conventional marker cannot find such precondition to diabetes, for example, the risk can be used as an item of examination for life insurance or the like.

[0025]

For quantitatively determining myo-inositol in a sample, 1 to 500 „L of the sample is added to the composition for myo-inositol quantitative determination to allow a reaction at 37°C and then the change in the amount of a coenzyme may be directly or indirectly determined for several minutes or several tens of minutes between two time points after the reaction starts, for example, the change for 1 minute between 3 minutes and 4 minutes after the reaction initiation or that for 5 minutes between 3 minutes and 8 minutes. In this case, the myo-inositol content in the sample can be determined by making a comparison with changes in absorbance which are measured for known concentrations of myo-inositol.

[0026]

The composition (reagent) for the quantitative determination need to contain at least an enzyme that acts on myo-inositol, and preferably it further contains a coenzyme.

In addition, a surfactant such as polyoxyethylene octylphenyl ether (OP-10) may be added to the present reagent as appropriate.

Furthermore, the present reagent is used in a form of a liquid product, a freeze-dried product, or a frozen product.

[0027]

For quantitatively determining myo-inositol in a sample, any type of methods using an enzyme to quantitatively determine myo-inositol may be used. The enzyme to be used in the present invention, which is capable of quantitatively determining myo-inositol, includes any enzymes that act on at least myo-inositol. Of those, however, myo-inositol dehydrogenase is preferable, and myo-inositol dehydrogenase derived from Flavobaterium sp. 671 (FERM BP-7323, hereinafter abbreviated as F.sp.671) is most preferable. In addition, preferably, the myo-inositol dehydrogenase to be used has as low as possible or no contamination by

substances that adversely affects coenzymes, or substances having the activity of decomposing coenzymes, for example, NADH oxidase which decomposes coenzymes in the reagent such as thio-NAD, NADH, etc.

The strain F.sp.671 is deposited on an international basis with the deposit number of FERM BP-7323 (date of deposit: October 12, 2000) at Patent Organism Depositary, the National Institute of Advanced Industrial Science and Technology, Independent Administrative Agency, located at Center 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan.

[0028]

For the detection of myo-inositol, any type of methods capable of detecting myo-inositol may be used. The methods include: a method using a visible light coloring reagent, for example, typically yellow coloring with thio-NAD, blue coloring with nitro blue tetrazolium (NBT), or red coloring with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT); a luminescence method; a fluorescence method; a method involving detection of an electric change; and a combination of these methods with amplification techniques.

In addition, using a compact device which can utilize any one of the above methods, the determination of urinary myo-inositol can be carried out non-invasively without restriction on time and place.

[0029]

The assay for determining the activity of myo-inositol dehydrogenase is as follows:

(1) Activity Assay

<Composition of Reaction Solution>

100 mM Tris buffer (pH 8.5)

20 mM myo-inositol (Sigma Co., Ltd.)

2 mM nicotinamide adenine dinucleotide (NAD) (Oriental Yeast Co., Ltd.)

5 U/ml diaphorase (Asahi Kasei Corporation)

0.025 % nitro blue tetrazolium (NBT; Wako Pure Chemical Industries, Ltd.)

1.5 % Triton-X100 (Wako Pure Chemical Industries, Ltd.)

One ml of the above reaction solution is added to a small test tube. After the reaction solution is incubated at 37°C for 5 minutes, 20 „l of an enzyme solution diluted by B times is added thereto and mixed to start the reaction. After the reaction exactly for 5 minutes, 2 ml of 0.1N HCl is added and mixed to stop the reaction. An absorbance at 550 nm is measured to obtain A1. In addition, the same reaction solution excluding myo-inositol is used to carry out a similar measurement to obtain the absorbance A0. The enzyme activity can be calculated from the following equation.

[0030]

[Formula 1]

$$U/ml = [(A1-A0)/18.3] \times [1/5] \times [3.02/0.02] \times B$$

Numerals in the equation represent the following meanings.

18.3: Molar absorption constant of NTB

5: Reaction time

3.02: Total volume of reaction solution

0.02: Volume of enzyme solution

B: Dilution factor of enzyme solution

The properties of myo-inositol dehydrogenase derived from the strain F.sp.671 are as follows:

[0031]

(2) Enzyme Activity

This enzyme produces inosose and a reduced coenzyme in the presence of at least myo-inositol and a coenzyme. The coenzyme includes nicotinamide adenine dinucleotides (hereinafter abbreviated as NADs) such as nicotinamide adenine dinucleotide (NAD), acetylpyridine adenine dinucleotide (acetyl-NAD), nicotinamide hypoxanthine dinucleotide (deamino-NAD), pyridine aldehyde adenine dinucleotide (aldehyde-NAD), nicotinamide adenine dinucleotide phosphate (NADP), thio-nicotinamide adenine dinucleotide (thio-NAD), and thio-nicotinamide adenine dinucleotide phosphate (thio-NADP).

Table 1 shows the ratio of relative activities on use of each coenzyme (as 100% when NAD is used as a coenzyme). The relative activities were determined with the coenzyme changed according to the following method.

[0032]

Relative Activity Assay

<Composition of Reaction Solution>

Buffer:	100 mM glycine buffer (pH 10.0)
Substrate:	20 mM myo-inositol (Sigma, Co., Ltd.)
Coenzyme:	2 mM
	(NAD, thio-NAD, NADP, thio NADP; Oriental Yeast Co., Ltd.)

One ml of the above reaction solution is added to a quartz cell. Then, the quartz cell is placed in a spectrophotometer adjusted at a temperature of 37°C. The cell is incubated for 5 minutes or more and then 20 „l of an enzyme solution of about 1.0 U/ml is added thereto and mixed. The initial velocity is obtained from an absorbance change per minute at a

wavelength peculiar to each reduced coenzyme. The initial velocity obtained with each coenzyme is compared with the initial velocity (100%) obtained using NAD as a coenzyme to provide the relative activity.

[0033]

Table 1

Relative activity ratio for each coenzyme used

Name of bacterial strain	F.sp.671
Coenzyme	Myo-inositol
NAD	100 %
NADP	8 %
Thio-NAD	29 %
Thio-NADP	0 %

[0034]

(3) Substrate Specificity

According to the relative activity assay described above, the measurement was performed using the same concentration of D-chiro-inositol, D-mannose, D-fructose, D-galactose, mannitol, epi-inositol, or scyllo-inositol in place of the substrate in the reaction solution. Table 2 shows the enzyme activity for each substrate referring to the initial velocity of the reaction to myo-inositol as 100%. It is clear that the enzyme derived from the strain F.sp.671 is a dehydrogenase having high specificity to myo-inositol.

The substrates used include D-mannose, D-fructose, D-galactose, mannitol, D-chiro-inositol (as above: Wako Pure Chemical Industries, Ltd.), myo-inositol, epi-inositol, and scyllo-inositol (as above: Sigma, Co., Ltd.).

[0035]

Table 2

Substrate specificity

Name of bacterial strain	F.sp.671
Coenzyme	NAD
myo-Inositol	100 %
chiro-Inositol	18 %
scyllo-Inositol	less than 1 %
epi-Inositol	2 %
Galactose	less than 1 %
Fructose	less than 1 %
Mannose	less than 1 %
Mannitol	0 %

[0036]

(4) Optimum pH

Following the relative activity assay described above, the measurement was performed using each of 100 mM tris buffer (pH 7.0-9.0) and 100 mM glycine buffer (pH 9.0-11.0) in place of 100 mM of pH 10.0 glycine buffer in the reaction solution. The measurement showed that the optimum pH was about 11.0 (substrate: myo-inositol).

[0037]

(5) Molecular Weight

Used were TSK gel G300SW (0.75 „ x 600 mm), eluent: 50 mM phosphate buffer (pH 7.5) +0.2 M Na_2SO_4 + 0.05% NaN_3, a molecular weight marker set of Oriental Yeast Co., Ltd. (Japan), and a chromatography apparatus made by Shimadzu Corporation (Japan). For the detection, the absorbance at UV 280 nm and the activity of each fraction were measured. myo-Inositol was used as a substrate in the activity measurement, revealing the molecular weight of 40,000 ± 10,000.

[0038]

(6) Heat Stability

The enzyme showed almost 100% remaining activity after treatment at 40°C for 15 minutes. The enzyme solution of about 5 U/ml was subjected to heat treatment for 15 minutes. The remaining activity was measured using the enzyme activity assay described above. In the activity measurement, myo-inositol was used as a substrate.

[0039]

(7) Km value

Using the relative activity assay described above, the concentration of myo-inositol, and the concentrations of NAD and thio-NAD were changed to determine Km values respectively. Using the activity assay described above, the substrate concentration was changed to calculate the Km value.

Km value for substrate

myo-Inositol: 1.7 ± 0.2 mM

Km value for coenzyme

NAD: 0.04 ± 0.01 mM

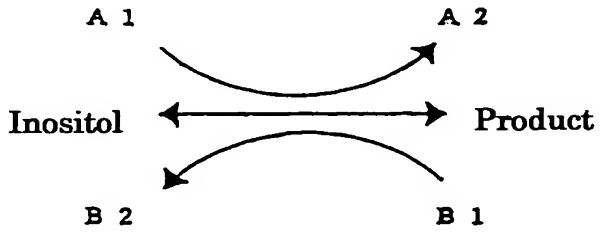
Thio NAD: 4.5 ± 1 mM

[0040]

For the quantitative determination of myo-inositol with higher sensitivity, the enzymatic cycling method can be used. An example of the enzymatic cycling method is illustrated in

the following equation.

[Chemical Formula 1]



[0041]

In the equation, A1 represents NAD(P) or thio-NAD(P); A2 represents a reduced form of A1; B1 represents reduced NAD(P) when A1 is thio-NAD(P) or reduced thio-NAD(P) when A1 is NAD(P); and B2 represents an oxidized product of B1. As used herein, NAD(P) represents nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate.

[0042]

For the solution composition of the quantitative reaction of myo-inositol using the enzymatic cycling, two or more of coenzymes are appropriately selected in view of Km values of respective coenzymes of myo-inositol dehydrogenase, and subsequently the pH condition is adjusted between the optimal pH values of forward reaction/reverse reaction to make an efficient progress in the enzymatic cycling. The amounts of A1 and B1 should be excess over the myo-inositol content in a sample and also excess in light of Km values of myo-inositol dehydrogenase for A1 and B1.

When using, for instance, myo-inositol dehydrogenase derived from F.sp.671, the Km values for NAD and thio-NAD are 0.04 mM and 4.5 mM, respectively. For the cycling reaction, thio-NAD and NADH may be selected as coenzymes. The concentrations of A1 and B1 are preferably 0.02 mM to 2 M, particularly preferably 0.05 to 100 mM. The amount of myo-inositol dehydrogenase is preferably 1 to 1000 U/mL, particularly preferably 1 to 100 U/mL. The amounts can be suitably selected on the basis of type and amount of the test sample, the myo-inositol content in the sample to be assayed, and the like; but other amounts may be also allowed.

[0043]

When hexokinase is used as an enzyme for eliminating sugars presented in a sample, any hexokinase capable of catalyzing the reaction from glucose to glucose-6-phosphate may be used, including hexokinase derived from Bacillus sp. Preferable hexokinase is one having excellent heat stability. The hexokinase having excellent heat stability can be obtained by

the method described in "Stable Hexokinase and Production Method Thereof" (JP 2000-078982 A).

[0044]

Because ADP generated together with glucose-6-phosphate has some inhibitory effect on the reaction in the enzymatic cycling method, the present inventors successfully have used ADP-dependent hexokinase simultaneously with hexokinase to improve substantially the elimination of glucose without any influence on the reaction of myo-inositol dehydrogenase.

Glc + ATP + Mg^{2+} ' G-6-P + ADP

Glc + ADP + Mg^{2+} ' G-6-P + AMP

ATP: Adenosine-5'-triphosphate

ADP: Adenosine-5'-diphosphate

AMP: Adenosine-5'-monophosphate

[0045]

The assay of hexokinase activity is conducted as follows.

<Composition of Reaction Solution>

50 mM Tris buffer (pH 8.5) (Sigma, Co., Ltd.)

20 mM glucose (Wako Pure Chemical Industries, Ltd.)

4 mM ATP (Oriental Yeast Co., Ltd.)

5 U/mL glucose-6-phosphate dehydrogenase (Toyobo Co., Ltd.)

1 mM NADP (Oriental Yeast Co., Ltd.)

10 mM magnesium chloride (Wako Pure Chemical Industries, Ltd.)

Solution for dissolving and diluting the enzyme: 50 mM Tris buffer (pH 8.5)

[0046]

One mL of the above reaction solution is added to a quartz cell with 1-cm optical path length, and incubated at 37°C for 5 minutes. Then, 20 „L of the enzyme solution, which is diluted B times, is added thereto and mixed to start the reaction. The absorbance at 340 nm is measured from the initiation of the reaction to obtain the absorbance change A1 per minute, while the absorbance indicates a linear change. A blind test is also conducted in a similar reaction to obtain the absorbance change A0 per minute, except that 50 „L of the solution for dissolving and diluting the enzyme is added instead of the enzyme solution. The enzyme activity is calculated from the following equation.

[0042]

[Formula 2]

U/ml = [(A1 – A0)/6.22] x [1.02/0.02] x B

Numerals in the equation represent the following meanings.

6.22: Millimolar absorbance constant of NADPH at 340 nm

1.02: Total volume of reaction solution (mL)

0.02: Volume of enzyme solution used in the reaction (mL)

B: Dilution factor of enzyme solution

[0048]

The assay of ADP-dependent hexokinase activity is conducted as follows.

<Composition of Reaction Solution>

50 mM Tris buffer (pH 7.5)

20 mM glucose solution (Wako Pure Chemical Industries, Ltd.)

2 mM ADP solution (pH 7.0) (Oriental Yeast Co., Ltd.)

5 U/mL glucose-6-phosphate dehydrogenase (Asahi Kasei Corporation)

1 mM NADP solution (Oriental Yeast Co., Ltd.)

2 mM magnesium chloride solution (Wako Pure Chemical Industries, Ltd.)

Solution for dissolving and diluting the enzyme: 10 mM Tris buffer (pH 7.5)

[0049]

Three mL of the above reaction solution is added to a small test tube, and incubated at 37°C for 5 minutes. Then, 50 „L of the enzyme solution, which is diluted by B times, is added thereto and mixed to start the reaction. The absorbance at 340 nm is measured from the initiation of the reaction to obtain the absorbance change A1 per minute, while the absorbance indicates a linear change. A blind test is also conducted in a similar reaction to obtain the absorbance change A0 per minute, except that 50 „L of the solution for dissolving and diluting the enzyme is added instead of the enzyme solution. The enzyme activity is calculated from the following equation.

[0050]

[Formula 3]

U/ml = [(A1 – A0)/6.22] x [3.05/0.05] x B

Numerals in the equation represent the following meanings.

6.22: Millimolar absorbance constant of NADPH at 340 nm

3.05: Total volume of reaction solution (mL)

0.05: Volume of enzyme solution used in the reaction (mL)

B: Dilution factor of enzyme solution

The amount of hexokinase is preferably 1 to 1,000 u/mL, particularly preferably 1 to 100

u/mL. The amount of ADP-dependent hexokinase is preferably 1 to 1,000 u/ml, particularly preferably 1 to 100 u/mL. The amounts can be appropriately selected depending on type and amount of the test sample, and other amounts can be also used.

[0051]

In addition, for the determination of urinary myo-inositol over a wide range of its concentration with good reproducibility, an enzymatic cycling reaction should be effectively performed. As a result of intensive examination on concentrations and ratio of thio-NAD and NADH, two coenzymes to be used in the enzymatic cycling reaction, the present inventors have found that the thio-NAD level is preferably 0.01 mM or more, particularly preferably 2 to 10 mM in a final concentration and the ratio of NADH/thio-NAD is preferably 0.01 to 0.5, particularly preferably 0.01 to 0.1. However, the amounts can be appropriately selected according to type and amount of the test sample, and other amounts may be applied.

[0052]

[Examples]

The examples of the present invention and reference examples will be described in detail, but the present invention is not limited thereto.

Reference Example 1 (Study on thio-NAD levels)

1) Reagents

<R-1>

5 mM MES (2-Morpholinoethanesulfonic acid) (pH 6.0)

0 to 40 mM thio-NAD (Oriental Yeast Co., Ltd.)

<R-2; Reagent for myo-inositol quantitative determination>

200 mM Bicine (pH 9.0)

0.3 mM NADH (Oriental Yeast Co., Ltd.)

25 u/mL myo-inositol dehydrogenase (Asahi Kasei Corporation)

2) Method

The measurement device used was Autoanalyzer 7170S (Hitachi Chemical Co., Ltd.). To 3 „L of myo-inositol solution at concentrations of 0 to 3,000 „M, 180 „L of R-1 reagent was added and incubated at 37°C for 4.8 minutes, followed by the addition of 180 „L of R-2 reagent to start the reaction. Absorbance at 405 nm was measured at 5.4 and 7.8 minutes after the reaction initiation, and then the difference therebetween was obtained. An increasing rate of absorbance per minute („mABS/min) was calculated and the sensitivity was then investigated with respect to the standard solution.

3) Results

The results are shown in Fig. 1. As shown in Fig. 1, using thio-NAD at final concentrations of 0.1 to 10 mM, the linearity of the calibration curve was observed for myo-inositol concentrations of 0 to 3,000 „M. In addition, it has been found that the final concentration of thio-NAD is preferably 2 to 10 mM to enhance the sensitivity of myo-inositol detection.

[0053]

Reference Example 2

(Study on buffers in reagents for myo-inositol quantitative determination)

1) Reagents

<R-1>

5 mM MES (pH 6.0)

5 mM thio-NAD (Oriental Yeast Co., Ltd.)

<R-2; Reagent for myo-inositol quantitative determination>

100 mM Buffer (pH 8.8)

0.5 mM NADH (Oriental Yeast Co., Ltd.)

10 u/mL myo-inositol dehydrogenase (Asahi Kasei Corporation)

2) Method

R-2 reagents for myo-inositol quantitative determination were prepared following the above shown formula with a buffer selected from:

Tris (Tris(hydroxymethyl)aminomethane),

Tricine (N-Tris(hydroxymethyl)-methylglycine),

Bicine (N,N-Bis(hydroxyethyl)glycine),

TAPS (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid),

TEA (Triethanolamine),

CHES (2-(Cyclohexylamino)ethanesulfonic acid), and

AMPSO(3-((1,1-Dimethyl-2-hydroxyethyl)amino)-2-hydroxypropanesulfonic acid).

The measurement device used was Autoanalyzer 7170S (Hitachi Chemical Co., Ltd.). To 15 „L of the standard 100-„M myo-inositol solution prepared in advance, 180 „L of R-1 reagent was added and incubated at 37°C for 4.8 minutes, followed by the addition of 60 „L of R-2 reagent to start the reaction. Absorbance at 405 nm was measured at 5.4 and 7.8 minutes after the reaction initiation, and then the difference therebetween was obtained. An increasing rate of absorbance per minute („mABS/min) was calculated and the sensitivity was then investigated with respect to the standard solution. The stability of each R-2 reagent was investigated by an acceleration test wherein R-2 reagent alone was stored in an incubator

at 30°C for 20 days and the same test as described above was conducted during the 20-day period on the 7th, 12th, and 20th days.

3) Results

The results are shown in Fig. 2. The buffers showing stable sensitivity in a reagent using the standard solution were Tris, Tricine, Bicine and TEA; and the buffer having the most stable sensitivity was Bicine.

[0054]

Reference Example 3 (Study on ADP-hexokinase)

1) Reagents

<R-1>

5 mM MES (pH 6.0)

5 mM $MgCl_2$ (Wako Pure Chemical Industries, Ltd.)

8 mM ATP (Oriental Yeast Co., Ltd.)

10 mM thio-NAD (Oriental Yeast Co., Ltd.)

10 u/mL ATP-hexokinase (Asahi Kasei Corporation)

0 to 4 u/mL ADP-hexokinase (Asahi Kasei Corporation)

<R-2; Reagent for myo-inositol quantitative determination>

200 mM Bicine (pH 9.0)

0.3 mM NADH (Oriental Yeast Co., Ltd.)

25 u/mL myo-inositol dehydrogenase (Asahi Kasei Corporation)

2) Method

The measurement device used was Autoanalyzer 7170S (Hitachi Chemical Co., Ltd.). The mixture of 100 „L of 2,000 „M myo-inositol solution and 1 mL of 0 to 10 g/dL glucose solution was prepared to be used as sample. To 3 „L of sample solution, 180 „L of R-1 reagent was added and incubated at 37°C for 4.8 minutes, followed by the addition of 180 „L of R-2 reagent to start the reaction. Absorbance at 405 nm was measured at 5.4 and 7.8 minutes after the reaction initiation, and then the difference therebetween was obtained. An increasing rate of absorbance per minute („ mABS/min) was calculated and the sensitivity was then investigated for respective samples.

3) Results

The results are shown in Fig. 3. By using ATP-hexokinase together with ADP-hexokinase, the increasing glucose level has little influence on the sensitivity and thus myo-inositol level can be determined more accurately.

[0055]

Reference Example 4

(myo-inositol quantitative determination with high sensitivity using enzyme)

1) Reagents

Reagent for myo-inositol assay

<R-1; Glucose eliminating reagent>

5 mM MES (pH 6.0)

0.05% NaN_3 (Wako Pure Chemical Industries, Ltd.)

0.05% OP-10 (Nippon Chemicals)

5 mM $MgC1_2$ (Wako Pure Chemical Industries, Ltd.)

8 mM ATP (Oriental Yeast Co., Ltd.)

10 mM thio-NAD (Oriental Yeast Co., Ltd.)

10 u/mL ATP-hexokinase (Asahi Kasei Corporation)

4 u/mL ADP-hexokinase (Asahi Kasei Corporation)

<R-2; Reagent for myo-inositol quantitative determination>

200 mM Bicine (pH 9.0)

0.05% NaN_3 (Wako Pure Chemical Industries, Ltd.)

40 mM $KHCO_3$ (Wako Pure Chemical Industries, Ltd.)

0.3 mM NADH (Oriental Yeast Co., Ltd.)

25 u/mL myo-inositol dehydrogenase (Asahi Kasei Corporation)

2) Method

The measurement device used was Autoanalyzer 7170S (Hitachi Chemical Co., Ltd.). To 3 „L of the myo-inositol solution prepared in advance, 180 „L of the glucose eliminating reagent was added and incubated at 37°C for 4.8 minutes to eliminate glucose, and then 180 „L of the reagent for myo-inositol quantitative determination was added thereto to start the reaction. Absorbance at 405 nm was measured at 5.4 and 7.8 minutes after the reaction initiation, and then the difference therebetween was obtained. An increasing rate of absorbance per minute („ mABS/min) was calculated.

3) Results

The results are shown in Fig. 4. As shown in Fig. 4, the present assay reagent allowed quantitative determination of myo-inositol in a simple way. The measurement range of myo-inositol was 0 to 2,000 „M and the lower limit of detection was 10 „M when the lower limit of detection was defined as the minimum concentration, which does not overlap "the mean of „ mABS/min + 2 x standard deviation" obtained by multiple measurements of 0 mM myo-inositol.

[0056]

Example 1

(Detection of mild impaired glucose tolerance by determination of urinary myo-inositol)

1) Subjects:

One hundred and twelve subjects were examined by the standard 75g oral glucose load test. Blood samples were collected just before the glucose load, and at 30, 60, 120, and 180 minutes after the glucose load to determine levels of blood glucose and insulin. Simultaneously, urine samples were collected just before the glucose load, and at 60, 120, and 180 minutes after the glucose load to determine levels of myo-inositol, urinary glucose, and creatinine.

2) Reagents and Assays:

Blood glucose: Electrode method (Kyoto Daiichi Kagaku Corporation: GA-1160)

Insulin: RIA2 Antibody method

myo-inositol assay reagent: the same as that of Example 4

Urinary glucose: Electrode method (Kyoto Daiichi Kagaku Corporation: GA-1160)

Creatinine: Creatinine-HA test Wako (Wako Pure Chemical Industries, Ltd.)

3) Method:

„PG, the total of blood glucose levels just before 75g oral glucose load, and at 30, 60, and 120 minutes after the glucose load, was used as an index of glucose tolerance. Myo-inositol level and creatinine level in respective urine samples just before the 75g oral glucose load, and at 30, 60, and 120 minutes after the glucose load were determined to calculate the myo-inositol amount to the amount of urinary creatinine excreted (myo-inositol/creatinine). In addition, „ myo-inositol content [(myo-inositol content at 60 min – myo-inositol content before load) /2] + [(myo-inositol content at 120 min – myo-inositol content before load) /2] was used as an index of myo-inositol level between before and after the glucose load. The relationship between „PG and „ myo-inositol content was investigated.

4) Results:

The results are shown in Table 3 and Fig. 5. As shown in Fig. 5, „PG and „ myo-inositol content showed a very good correlation. Higher „PG indicates that blood glucose levels are kept higher after the glucose load, and thus the presence of impaired glucose tolerance. In addition, for example, if the characteristic value of „ myo-inositol is set as 10 „g/mg Cre (creatinine), an effective detection can be made for cases having mild impaired glucose tolerance with „PG level of about 530 mg/dL.

[0057]

Table 3

		Blood glucose (mg/dL)					Insulin (μU/mL)					I.I	Urinary myo-Inositol (μg/mg Cre)					Urinary Glucose (g/dL)				Δ myo-Inositol	ΣPG	ΔIRI/ΔPG
		0 min.	30	60	120	180	ΣPG	0 min.	30	60	120	180		0 min.	60	120	180	Δmyo	0 min.	60	120	180			
1	Normal	89	150	161	135	94	535	5	23	23	22	13	0.30	13	24	33	26	15	0.03	0.02	0.02	0.01	15	535	0.30
2	Normal	105	160	153	85	107	483	6	59	40	24	22	0.96	23	24	40	26	9	0.02	0.01	0.01	0.01	9	483	0.98
3	Normal	102	119	158	121	76	500	2	26	47	20	11	1.41	11	12	13	13	1	0.01	0.02	0.02	0.02	1	500	1.41
4	Normal	85	170	143	98	85	496	5	41	76	21	14	0.42	46	60	51	43	8	0	0.07	0.01	0.01	8	496	0.42
5	Normal	106	164	162	115	83	547	5	17	19	20	8	0.21	13	13	19	15	3	0.02	0.03	0.03	0.02	3	547	0.21
6	Normal	96	125	139	134	125	494	5	10	24	25	16	0.17	28	38	59	68	23	0.01	0.01	0.01	0.01	23	494	0.17
7	Normal	82	130	139	80	78	451	5	16	13	19	11	0.28	21	24	32	29	7	0.01	0.02	0.02	0.01	7	451	0.28
8	Normal	101	144	135	108	107	488	7	37	27	36	34	0.70	24	17	23	22	-4	0.02	0.03	0.03	0.03	-4	488	0.70
9	Normal	87	161	172	109	60	529	3	25	21	17	4	0.30	25	35	46	44	16	0.01	0.03	0.01	0.01	16	529	0.30
10	Normal	106	175	167	133	60	581	13	83	124	107	14	1.01	17	26	35	25	14	0.02	0.01	0	0	14	581	1.01
11	Normal	106	158	129	128	72	521	12	63	50	73	11	0.98	9	16	14	14	6	0.01	0	0.01	0	6	521	0.98
12	Normal	101	137	101	83	82	422	11	87	86	25	10	2.11	9	12	20	13	7	0.01	0.01	0	0	7	422	2.11
13	Normal	98	129	81	101	97	409	7	49	10	18	11	1.35	13	23	18	20	8	0.01	0	0	0.01	8	409	1.35
14	Normal	92	127	123	85	70	427	9	39	25	18	8	0.86	11	12	11	12	0	0.02	0.01	0.01	0	0	427	0.86
15	Normal	95	132	103	102	69	432	8	39	30	28	12	0.84	9	12	11	11	3	0.01	0.01	0.01	0.01	3	432	0.84
16	Normal	108	167	107	102	85	482	10	75	32	21	9	1.07	9	11	12	12	2	0.03	0.03	0.02	0.02	2	482	1.07
17	Normal	88	121	138	100	79	447	6	18	32	15	5	0.36	9	10	15	17	4	0.01	0.01	0.01	0.01	4	447	0.36
18	Normal	97	130	88	103	102	418	5	28	18	14	8	0.70	14	13	11	11	-2	0.01	0.01	0.01	0.01	-2	418	0.70
19	Normal	99	161	114	105	108	469	7	7	8	24	16	0.00	8	10	12	18	3	0.02	0.02	0.02	0	3	469	0.00
20	Normal	102	148	178	127	89	553	8	62	59	38	20	1.17	41	70	82	47	40	0.01	0.24	0.4	0.03	40	553	1.17
21	Normal	89	108	120	92	102	409	10	51	65	21	37	2.16	35	34	40	31	2	0.01	0.01	0.01	0.01	2	409	2.16
22	Normal	91	144	153	105	72	493	9	75	67	38	7	1.25	28	28	25	17	-2	0.01	0.03	0.02	0.01	-2	493	1.25
23	Normal	101	154	148	91	73	494	5	28	36	14	5	0.40	7	8	8	9	1	0.02	0.02	0.02	0.01	1	494	0.40
24	Normal	98	148	177	124	52	545	10	29	59	67	10	0.37	11	11	11	11	0	0.02	0.03	0.02	0	0	545	0.37
25	Normal	95	168	98	84	76	445	6	78	20	16	4	0.99	24	34	30	31	8	0.01	0.02	0.01	0	8	445	0.99
26	Normal	89	147	93	84	55	413	5	28	23	21	4	0.38	7	9	14	8	5	0.02	0.01	0	0.01	5	413	0.38
27	Normal	90	129	83	101	81	413	7	36	14	17	6	0.74	25	22	20	19	-4	0	0.01	0.01	0	-4	413	0.74
28	Normal	75	96	75	84	60	330	3	37	17	13	4	1.82	11	13	13	14	2	0.03	0.03	0.02	0.02	2	330	1.82
29	Normal	92	127	87	83	68	389	5	37	25	11	5	0.91	25	21	20	18	-4	0.03	0.01	0	0.01	-4	389	0.91
30	Normal	83	109	107	101	78	400	9	32	27	26	12	0.88	16	15	17	14	0	0	0.01	0	0	0	400	0.88
31	Normal	100	123	71	89	79	383	7	32	33	13	3	1.09	5	8	13	10	5	0.03	0	0	0	5	383	1.09
32	Normal	96	110	75	89	82	370	6	46	20	18	7	2.88	15	18	17	12	2	0.01	0.01	0	0.02	2	370	2.86
33	Normal	91	108	82	80	82	361	7	16	17	19	8	0.53	10	13	16	15	4	0.02	0.01	0	0	4	361	0.53
34	Normal	91	140	152	89	59	482	3	2	5	7	3	-0.02	7	8	8	7	1	0.01	0.01	0.01	0	1	482	-0.02
35	Normal	78	119	120	89	80	407	4	9	5	7	2	0.13	10	12	15	14	4	0.02	0.01	0.01	0.01	4	407	0.13
36	Normal	100	151	148	139	100	538	10	41	45	49	27	0.61	18	26	38	36	14	0.02	0.03	0.03	0	14	538	0.61
37	Normal	96	119	110	85	63	410	5	38	34	18	5	1.43	20	22	28	29	5	0.01	0.01	0	0	5	410	1.43
38	Normal	99	145	149	109	63	602	9	21	31	17	7	0.26	23	36	40	32	15	0.01	0.02	0.01	0	15	502	0.26
39	Normal	85	98	101	82	50	366	4	14			4	0.77	8	8	8	9	0	0.01	0.01	0.02	0	0	366	0.77

Table 3 (continuation)

40	Normal	89	114	87	91	63	381	7	9	10	15	3	0.08	27	35	44	44	12	0.01	0.02	0.01	0	12	381	0.08
41	Normal	95	169	175	109	59	548	11	35	46	29	7	0.32	9	10	9	9	0	0	0.02	0.01	0.01	0	548	0.32
42	Normal	76	78	66	90	84	310	2	7	4	10	5	2.50	8	8	8	8	−1	0.01	0.01	0.02	0.01	−1	310	2.50
43	Normal	100	124	92	87	65	403	8	51	29	18	5	1.79	8	7	9	12	1	0.01	0.03	0.01	0	1	403	1.79
44	Normal	95	138	117	91	67	441	7	26	44	27	6	0.44	10	17	23	26	10	0.02	0	0	0	10	441	0.44
45	Normal	89	106	85	82	79	362	6	79	32	16	4	4.29	7	8	9	12	2	0.02	0.01	0.01	0	2	362	4.29
46	Normal	103	192	153	96	80	544	9	33	44	20	5	0.27	18	56	38	28	28	0.02	0.77	0.06	0	28	544	0.27
47	Normal	87	131	123	109	69	450	6	42	36	24	4	0.82	7	10	10	9	3	0.01	0.01	0.01	0.01	3	450	0.82
48	Normal	99	126	104	83	66	412	10	47	9	15	5	1.37	27	18	21	18	−7	0	0.02	0	0.01	−7	412	1.37
49	Normal	86	106	86	85	89	363	3	22	18	17	8	0.95	14	20	19	14	5	0	0	0	0	5	363	0.95
50	Normal	84	131	89	89	80	393	6	40	24	12	10	0.72	10	15	17	16	5	0.03	0	0	0	5	393	0.72
51	Normal	85	116	99	110	83	410	6	17	17	18	9	0.35	13	13	12	11	−1	0.03	0.02	0.02	0	−1	410	0.35
52	Normal	87	122	89	96	88	394	6	27	30	35	13	0.80	11	16	20	24	7	0.02	0.02	0.01	0	7	394	0.80
53	Normal	90	147	112	116	52	465	6	72	53	50	8	1.16	17	16	22	26	2	0	0	0	0	2	465	1.16
54	Normal	92	187	186	133	49	598	3	17	25	27	4	0.15	368	487	623	395	186	0	0.23	0.15	0.03	186	598	0.15
55	Normal	99	146	181	133	137	559	5	19	16	15	13	0.30	4	4	4	3	1	0	0	0.01	0	1	559	0.30
56	Normal	99	160	206	138	68	603	11	30	49	72	18	0.31	7	7	14	10	4	0.03	0.04	0	0	4	603	0.31
57	Normal	107	151	189	115	44	562	6	16	44	38	7	0.23	13	20	42	30	18	0.02	0.03	0.05	0.02	18	562	0.23
58	Normal	105	194	234	110	81	643	5	17	40	23	11	0.13	24	32	72	29	28	0	0.04	0.13	0	28	643	0.13
59	Normal	89	167	187	130	108	573	22	133	142	104	79	1.42	26	34	41	27	12	0.02	0.02	0.01	0	12	573	1.42
60	IFG	117	165	154	90	80	526	8	37	108	27	11	0.60	24	46	72	45	35	0.01	0.06	0.06	0	35	526	0.60
61	IFG	115	174	206	136	65	631	4	8	19	20	4	0.07	12	33	84	25	46	0.01	0.23	0.55	0	46	631	0.07
62	IFG	117	204	220	103	152	644	7	31	37	33	16	0.28	37	113	140	56	90	0.01	0.61	1.04	0.12	90	644	0.28
63	IFG	110	185	240	127	120	662	6	15	26	15	14	0.12	17	26	59	29	26	0.02	0.05	0.12	0.03	26	662	0.12
64	IFG	111	183	200	83	75	577	4	16	22	12	4	0.17	16	26	48	28	22	0	0.1	0.17	0	22	577	0.17
65	IFG	110	153	60	88	82	411	5	44	22	18	15	0.91	13	15	19	19	4	0.01	0.01	0.02	0.01	4	411	0.91
66	IFG	117	236	192	101	71	646	24	196	100	48	17	1.45	12	19	19	13	7	0.02	0.03	0.02	0.01	7	646	1.45
67	IFG	111	166	167	124	70	568	12	53	55	40	8	0.75	9	22	21	14	13	0.01	0.02	0.03	0	13	568	0.75
68	IFG	113	204	229	115	58	661	5	20	47	44	12	0.16	49	111	96	42	54	0.01	0.89	0.53	0.03	54	661	0.16
69	IGT	111	192	237	161	63	701	10	18	26	48	9	0.10	22	67	146	94	85	0.01	0.37	1.31	0.41	85	701	0.10
70	IGT	104	153	130	141	141	528	7	28	24	17	16	0.43	15	22	29	28	10	0.02	0.02	0	0.01	10	528	0.43
71	IGT	113	195	224	170	147	702	7	19	44	48	31	0.15	63	114	185	176	86	0.01	0.67	0.38	0.14	86	702	0.15
72	IGT	98	158	213	143	48	612	5	14	22	33	5	0.15	59	72	149	75	52	0.03	0.19	1.16	0.17	52	612	0.15
73	IGT	95	119	147	142	73	503	5	20	21	29	6	0.63	40	31	36	40	−6	0.01	0.01	0.03	0.01	−6	503	0.63
74	IGT	103	187	217	164	111	671	6	18	30	35	11	0.14	21	55	74	27	44	0.01	0.02	0.01	0.01	44	671	0.14
75	IGT	123	196	232	150	158	701	3	30	60	67	56	0.37	31	88	119	88	72	0.01	0.95	0.53	0.66	72	701	0.37
76	IGT	108	150	192	168	124	618	3	17	22	32	26	0.33	15	14	15	13	0	0.02	0.02	0.02	0.02	0	618	0.33
77	IGT	114	178	204	172	136	668	4	10	13	17	12	0.09	29	47	89	49	39	0.02	0.04	0.13	0.03	39	668	0.09
78	IGT	108	211	214	167	69	700	6	36	50	41	10	0.29	15	66	116	28	76	0.01	0.22	0.43	0.03	76	700	0.29
79	IGT	97	211	256	196	117	760	6	20	28	34	16	0.12	15	29	29	26	15	0.03	0.08	0.05	0.01	15	760	0.12
80	IGT	123	218	244	160	83	745	2	11	13	21	7	0.09	17	73	150	27	95	0.02	1.41	1.54	0.02	95	745	0.09

Table 3 (continuation)

81	IGT	100	161	204	173	101	638	7	9	12	14	12	0.03	15	15	21	18	3	0.02	0.02	0.03	0.02	3	638	0.03
82	IGT	110	219	240	186	100	765	8	15	21	31	13	0.08	32	117	210	178	131	0.03	3.12	4.13	1.44	131	765	0.08
83	Diabetic	127	208	260	178	125	781	8	21	25	37	21	0.16	15	14	36	27	11	0.02	0.03	0.14	0.08	11	781	0.16
84	Diabetic	128	249	279	214	89	870	6	22	25	31	15	0.13	50	119	221	144	120	0.02	1.8	1.33	0.31	120	870	0.13
85	Diabetic	151	228	263	256	166	898	5	12	13	16	7	0.09	50	152	282	208	157	0.03	0.85	2.33	1.02	157	898	0.09
86	Diabetic	170	278	326	322	197	1096	5	7	8	11	9	0.02	64	146	232	225	125	0.01	1.33	3.46	2.75	125	1096	0.02
87	Diabetic	204	281	337	363	286	1185	12	15	19	26	28	0.04	21	63	145	151	83	0.05	3.42	7.25	7.25	83	1185	0.04
88	Diabetic	112	209	282	237	91	820	4	10	18	15	7	0.06	33	108	195	112	117	0.01	1.17	0.98	0.16	117	820	0.06
89	Diabetic	129	232	288	225	122	874	4	11	15	10	7	0.07	14	71	170	80	108	0.03	2.82	5.89	1.16	108	874	0.07
90	Diabetic	106	171	253	243	115	773	4	9	20	32	14	0.08	27	44	135	79	63	0.02	0.99	5.11	1.98	63	773	0.08
91	Diabetic	110	203	232	201	105	746	6	41	43	46	14	0.38	16	83	112	90	82	0.02	1.57	1.92	1.26	82	746	0.38
92	Diabetic	139	225	286	326	245	978	4	5	8	10	8	0.01	36	86	191	202	103	0.05	2.71	6.43	6.73	103	978	0.01
93	Diabetic	118	232	285	232	114	885	7	16	20	31	15	0.07	16	50	155	62	87	0.02	1.09	0.73	0.11	87	885	0.07
94	Diabetic	132	220	329	318	223	999	30	48	80	105	74	0.22	38	40	244	167	104	0.03	0.12	0.4	0.61	104	999	0.22
95	Diabetic	102	178	260	250	122	780	2	9	13	38	16	0.09	18	26	148	91	69	0	0.08	3.31	1.16	69	780	0.09
96	Diabetic	173	239	352	311	201	1075	9	7	23	36	20	-0.03	49	108	238	227	123	0.04	2.21	6.46	4.65	123	1075	-0.03
97	Diabetic	162	236	224	284	263	878	6	17	14	12	11	0.13	40	62	148	167	64	0.01	0.15	0.18	0.11	64	878	0.13
98	Diabetic	166	252	306	274	205	998	3	11	17	20	10	0.09	15	71	161	114	100	0.01	1.38	4.5	1.88	100	998	0.09
99	Diabetic	101	249	373	444	342	1167	3	5	7	6	6	0.01	37	154	280	301	185	0.02	3.44	2.45	2.1	185	1167	0.01
100	Diabetic	152	239	314	299	162	1004	7	11	33	31	12	0.05	33	58	174	127	83	0.03	0.36	3.28	2.23	83	1004	0.05
101	Diabetic	141	208	261	189	138	799	3	4	7	11	8	0.01	28	66	154	67	82	0.02	0.12	0.66	0.02	82	799	0.01
102	Diabetic	136	236	262	225	209	859	7	14	18	20	10	0.07	73	160	288	181	141	0.01	0.92	1.76	0.53	141	859	0.07
103	Diabetic	101	128	193	206	138	628	6	15	25	58	46	0.33	13	19	46	28	20	0.02	0	0.02	0.02	20	628	0.33
104	Diabetic	110	198	241	257	151	806	5	24	32	55	31	0.22	45	55	99	118	32	0.01	0.15	1.3	1.61	32	806	0.22
105	Diabetic	152	253	346	291	158	1042	10	19	44	16	23	0.09	99	183	371	280	178	0.11	3.02	3.32	3.2	178	1042	0.09
106	Diabetic	129	239	202	130	86	700	5	39	65	24	6	0.31	24	69	67	40	44	0.03	1.76	0.59	0.12	44	700	0.31
107	Diabetic	112	200	250	283	246	845	8	11	15	29	27	0.08	33	66	157	178	78	0.01	0.42	1.82	2.5	78	845	0.08
108	Diabetic	111	198	251	254	171	814	5	17	20	32	21	0.14	17	43	180	167	94	0.02	0.48	0.99	0.84	94	814	0.14
109	Diabetic	137	198	258	232	141	825	5	11	30	21	8	0.10	27	105	177	138	114	0.08	3.88	6.04	3.78	114	825	0.10
110	Diabetic	134	201	276	233	151	844	5	13	19	20	11	0.12	22	43	138	85	68	0.02	0.65	4.88	2.69	68	844	0.12
111	Diabetic	111	169	240	201	101	721	4	12	28	28	10	0.14	27	63	132	80	70	0.01	0.43	1.44	0.1	70	721	0.14
112	Diabetic	138	201	200	137	141	674	7	8	10	20	13	0.02	28	35	38	31	11	0.01	0.01	0	0	11	674	0.02

[0058]

Example 2

(Detection of Impaired early insulin secretion by Determination of Urinary myo-Inositol)

1) Subjects:

The same as those of Example 5.

2) Reagents and Assays:

The same as those of Example 5.

3) Method:

Myo-inositol levels and creatinine levels in each urine sample just before the 75g oral glucose load, and at 60 and 120 minutes after the glucose load were determined, and then the myo-inositol amount to the amount of urinary creatinine excreted (myo-inositol/creatinine) was obtained. In addition, „ myo-inositol content [(myo-inositol content at 60 min – myo-inositol content before load) /2] + [(myo-inositol content at 120 min – myo-inositol content before load) /2] was used as the index of myo-inositol level between before and after the glucose load. The relationship between the insulinogenic index (I.I) and „ myo-inositol content was investigated.

4) Results:

The results are shown in Fig. 6. As shown in Fig. 6, the relationship between the insulinogenic index and „ myo-inositol content was found. In a large percentage of cases where the „ myo-inositol content was 15 „g/mg Cre or more, the insulinogenic index showed less than 0.4. According to the guideline of the Japan Diabetes Society, the insulinogenic index of less than 0.4 can judge the presence of impaired early insulin secretion . As is evident from Fig. 6, if the characteristic value of „ myo-inositol is set at 15 „g/mg Cre, the cases showing the insulinogenic index of less than 0.4, or having impaired early insulin secretion , can be detected efficiently.

[0059]

Example 3

(Detection of mild impaired glucose tolerance by determination of urinary myo-inositol and urinary glucose)

1) Subjects:

The same as those of Example 5.

2) Reagents and Assays:

The same as those of Example 5.

3) Method:

Myo-inositol levels and creatinine levels in each urine sample just before the 75g oral glucose load, and at 60 and 120 minutes after the glucose load were determined. Then, the myo-inositol content to the amount of urinary creatinine excreted (myo-inositol/creatinine) was obtained. At the same time, urinary glucose level was also determined. „ myo-inositol content [(myo-inositol content at 60 min – myo-inositol content before load) /2] + [(myo-inositol content at 120 min – myo-inositol content before load) /2] was used as the index of myo-inositol level between before and after the glucose load.

4) Results

The case showing „ myo-inositol content of 10,,g/mg Cre or more was referred to as plus (+), while the other case was referred to as minus (-). Likewise for the urinary glucose, the case showing an urinary glucose level of 50 mg/dL or more at 2 hours after glucose load was referred to as "+", while the other case was referred to as "-". „PG values calculated in Example 5 were used.

Out of 112 subjects tested, 52 subjects were in the group of „ myo-inositol (-) and urinary glucose (-), 12 subjects were in the group of „ myo-inositol (+) and urinary glucose (-), and 48 subjects were in the group of „ myo-inositol (+) and urinary glucose (+). Nobody corresponded to the group of „ myo-inositol (-) and urinary glucose (+). The respective „PG values of the group of „ myo-inositol (-) and urinary glucose (-), the group of „ myo-inositol (+) and urinary glucose (-), and the group of „ myo-inositol (+) and urinary glucose (+) were compared with one another.

The results are shown in Fig. 7. As shown in Fig. 7, the mean and standard deviation of „PG values of the group of „ myo-inositol (-) and urinary glucose (-) were 453 mg/dL and 76.6 mg/dL, respectively. The mean and standard deviation of „PG values of the group of „ myo-inositol (+) and urinary glucose (-) were 556 mg/dL and 81.1 mg/dL, respectively. The mean and standard deviation of „PG values of the group of „ myo-inositol (+) and urinary glucose (+) were 791 mg/dL and 164.8 mg/dL, respectively. Furthermore, as compared with „PG of the group of „ myo-inositol (-) and urinary glucose (-), „PG of the group of „ myo-inositol (+) and urinary glucose (-) was significantly higher, and also „PG of the group of „ myo-inositol (+) and urinary glucose (+) was significantly much higher.

These show the degree of impaired glucose tolerance could be determined non-invasively by determining urinary myo-inositol and urinary glucose in combination.

[0060]

Example 4

(Detection of impaired early insulin secretion by determination of urinary myo-inositol and

urinary glucose)

1) Subjects:

The same as those of Example 5.

2) Reagents and Assays:

The same as those of Example 5.

3) Method:

The same as that of Example 7

4) Results:

The case showing ,, myo-inositol content of 10,,g/mg Cre or more was referred to as plus (+), while the other case was referred to as minus (-). Likewise for the urinary glucose, the case showing an urinary glucose level of 50 mg/dL or more at 2 hours after glucose load was referred to as (+), while the other case was referred to as (-). The insulinogenic index values calculated in Example 6 were used.

The respective insulinogenic index values (,, IRI_{30-0} / ,, PG_{30-0}) of the group of ,, myo-inositol (-) and urinary glucose (-), the group of ,, myo-inositol (+) and urinary glucose (-), and the group of ,, myo-inositol (+) and urinary glucose (+) were compared with one another.

The results are shown in Fig. 8. As shown in Fig. 8, the mean and standard deviation of,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (-) and urinary glucose (-) were 1.32 and 0.79, respectively. The mean and standard deviation of,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (+) and urinary glucose (-) were 0.45 and 0.42, respectively. The mean and standard deviation of,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (+) and urinary glucose (+) were 0.16 and 0.19, respectively. That is, as compared with,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (-) and urinary glucose (-),,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (+) and urinary glucose (-) was significantly lower, and,, IRI_{30-0} / ,, PG_{30-0} of the group of ,, myo-inositol (+) and urinary glucose (+) was significantly much lower.

These show that the degree of impaired early insulin secretion could be determined non-invasively by determining urinary myo-inositol and urinary glucose in combination.

[0061]

Example 5

(Detection of mild impaired glucose tolerance by determination of urinary myo-inositol)

1) Subjects:

Out of the subjects of Example 1, 59 subjects judged as normal glucose tolerance showing the fasting blood glucose level of less than 110 mg/dl and the two-hour postload glucose level of less than 140 mg/dl.

2) Regents and assays:

The same as those of Example 1.

3) Method:

Out of 59 subjects judged as normal glucose tolerance, those having the one-hour post load glucose level of 180 mg/dL or more or the two-hour post load glucose level of 120 mg/dL or more were referred to as B group or cases showing slightly decreased glucose tolerance (mild impaired glucose tolerance) and the others were referred to as A group. Fifty nine subjects of normal glucose tolerance included 45 subjects of A group and 14 subjects of B group. The myo-inositol content to the amount of urinary creatinine excreted (myo-inositol/creatinine) in A or B group was obtained by determining myo-inositol levels and creatinine levels in each urine sample just before 75g oral glucose load, and at 60 and 120 minutes after the glucose load. In addition, „ myo-inositol content [(myo-inositol content at 60 min – myo-inositol content before load) /2] + [(myo-inositol content at 120 min – myo-inositol content before load) /2] was used as the index of myo-inositol level between before and after the glucose load.

4) Results:

The results are shown in Fig. 9. As shown in Fig. 9, the mean of „ myo-inositol content of A group was 3.9 „g/mg Cre and the mean of „ myo-inositol content of B group was 25.8 „g/mg Cre. As compared with A group, B group (mild impaired glucose tolerance) with slightly decreased glucose tolerance resulted in higher „ myo-inositol content.

[0062]

Example 6

(Detection of insulin secretory defect by determination of urinary myo-inositol)

1) Subjects:

The same as those of Example 5.

2) Reagents and Assays:

The same as those of Example 1.

3) Method:

Out of subjects judged as normal glucose tolerance, those having the insulinogenic index of less than 0.4 were referred to as B group and the others were referred to as A group. Fifty nine subjects judged as normal glucose tolerance included 37 subjects of A group and 22 subjects of B group. The myo-inositol content to the amount of urinary creatinine excreted (myo-inositol/creatinine) in A or B group was obtained by determining myo-inositol levels and creatinine levels in each urine sample just before 75g oral glucose load, and at 60 and 120

minutes after the glucose load.

In addition, „ myo-inositol content [(myo-inositol content at 60 min – myo-inositol content before load) /2] + [(myo-inositol content at 120 min – myo-inositol content before load) /2] was used as the index of myo-inositol level between before and after the glucose load.

4) Results

The results are shown in Fig. 10. As shown in Fig. 10, the mean of „ myo-inositol content of A group was 4.4 „g/mg Cre and the mean of „ myo-inositol content of B group was 16.9 „g/mg Cre. As compared with A group, B group with impaired early insulin secretion resulted in higher „ myo-inositol content.

[0063]

[Effect of the Invention]

As described above, the present invention provides a method of detecting mild impaired glucose tolerance and/or impaired early insulin secretion in a non-invasive and convenient manner with good reproducibility.

[Brief Description of the Drawings]

[Fig. 1] Fig. 1 shows the results of study on thio-NAD levels based on Reference Example 1.

[Fig. 2] Fig. 2 shows the results of stability test of myo-inositol assay reagents based on Reference Example 2.

[Fig. 3] Fig. 3 shows the effects of ADP-hexokinase accordinormal glucose toleranceo Reference Example 3.

[Fig. 4] Fig. 4 shows the calibration curve for myo-inositol levels based on Reference Example 4.

[Fig. 5] Fig. 5 shows the relationship between myo-inositol level and „PG based on Example 1.

[Fig. 6] Fig. 6 shows the relationship between inositol level and insulinogenic index based on Example 2.

[Fig. 7] Fig. 7 shows the relationship between each group and „PG based on Example 3.

[Fig. 8] Fig. 8 shows the relationship between each group and insulinogenic index based on Example 4.

[Fig. 9] Fig. 9 shows the relationship between each group and myo-inositol based on Example 5.

[Fig. 10] Fig. 10 shows the relationship between each group and myo-inositol based on Example 6.

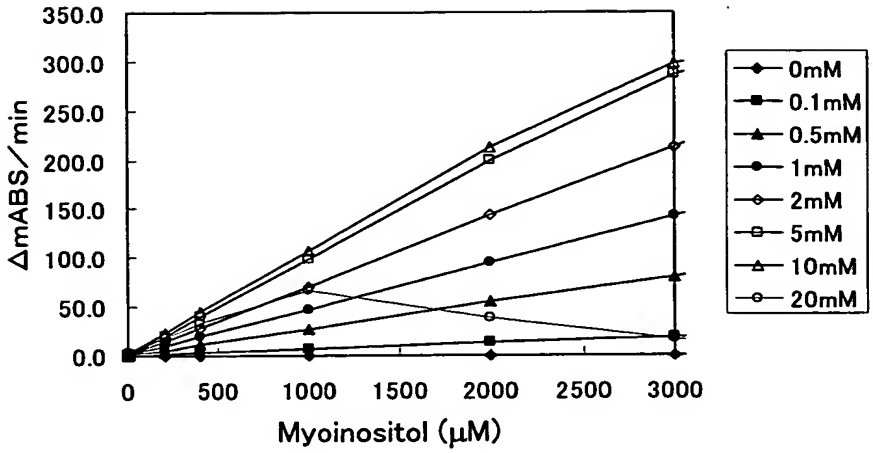
[Name of document] Abstract

[Problem] Providing a noninvasive method of conveniently detecting mild impaired glucose tolerance and/or impaired early insulin secretion which the use of an enzyme.

[Means to solve the problem] Mild impaired glucose tolerance and/or impaired early insulin secretion are detected by quantifying myoinositol secreted into the urine before loading glucose and after loading glucose for a definite period of time with the use of a reagent and comparing the increase (or the increase ratio) in the myoinositol content thus measured with a characteristic value which has been preliminarily determined in normal subjects.

[Selected drawing] None.

Fig. 1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300.0
250.0
200.0
150.0
100.0
50.0
0.0
ΔmABS/min
0
500
1000
1500
2000
2500
3000
Myoinositol (μM)
0mM
0.1mM
0.5mM
1mM
2mM
5mM
10mM
20mM

Fig. 2

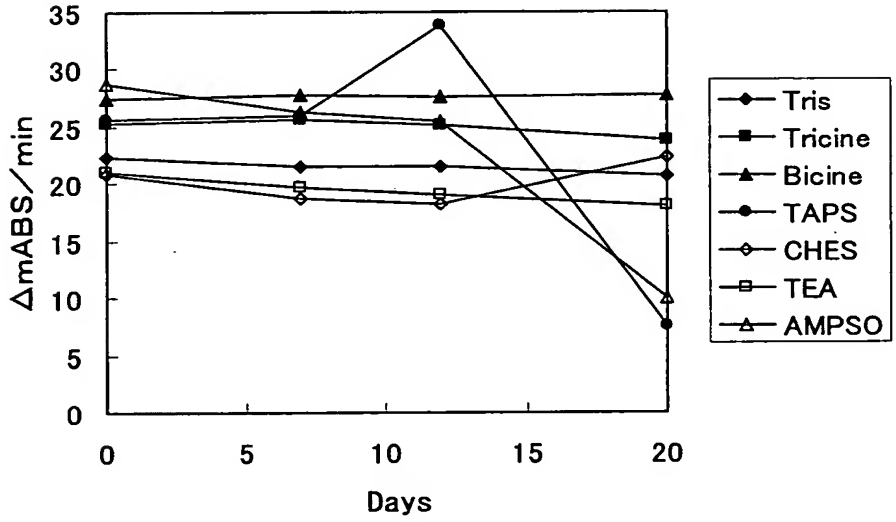

35
30
25
20
15
10
5
0
ΔmABS/min
0
5
10
15
20
Days
Tris
Tricine
Bicine
TAPS
CHES
TEA
AMPSO

Fig. 3

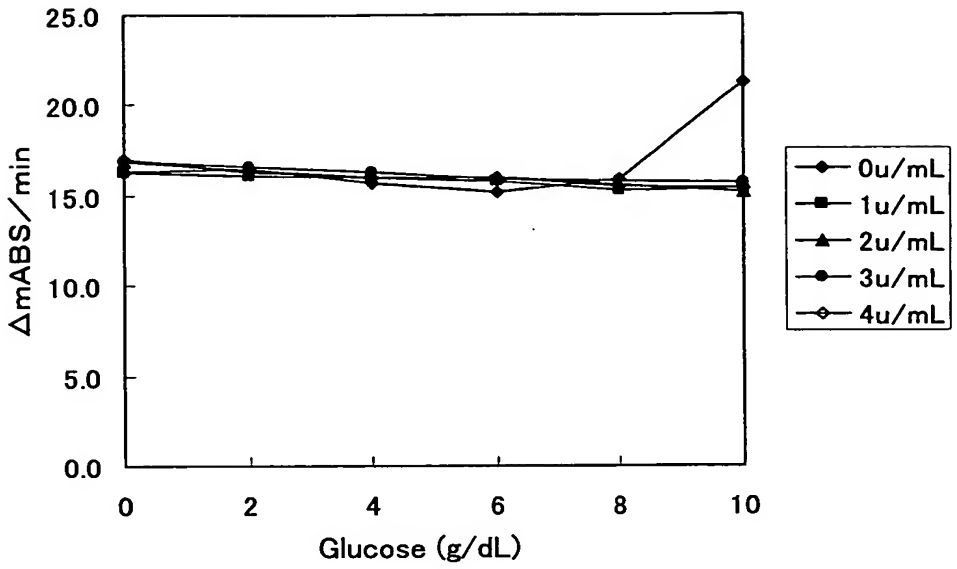

25.0
20.0
15.0
10.0
5.0
0.0
ΔmABS/min
0
2
4
6
8
10
Glucose (g/dL)
0u/mL
1u/mL
2u/mL
3u/mL
4u/mL

Fig. 4

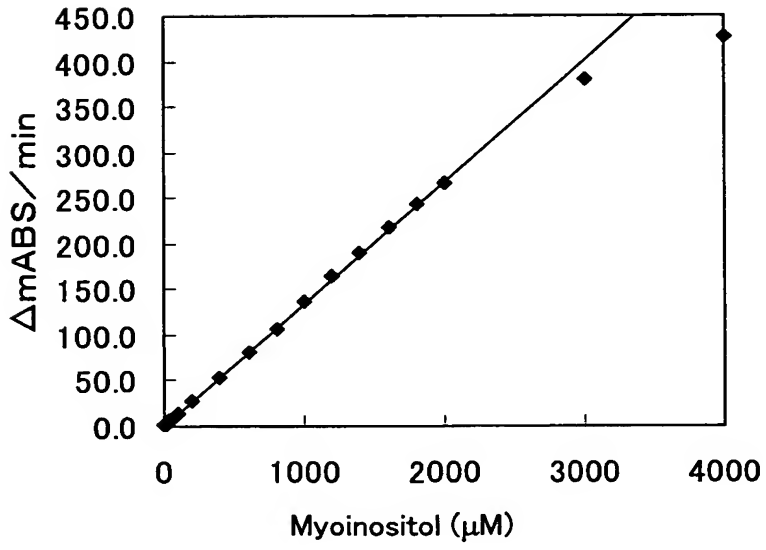

450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
ΔmABS/min
0
1000
2000
3000
4000
Myoinositol (μM)

Fig. 5

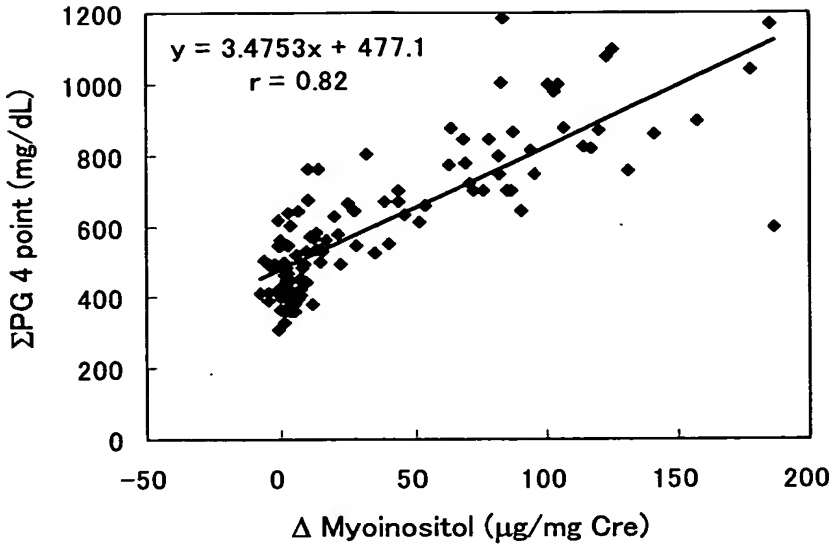

y = 3.4753x + 477.1
r = 0.82
ΣPG 4 point (mg/dL)
Δ Myoinositol (μg/mg Cre)
1200
1000
800
600
400
200
0
-50
0
50
100
150
200

Fig. 6

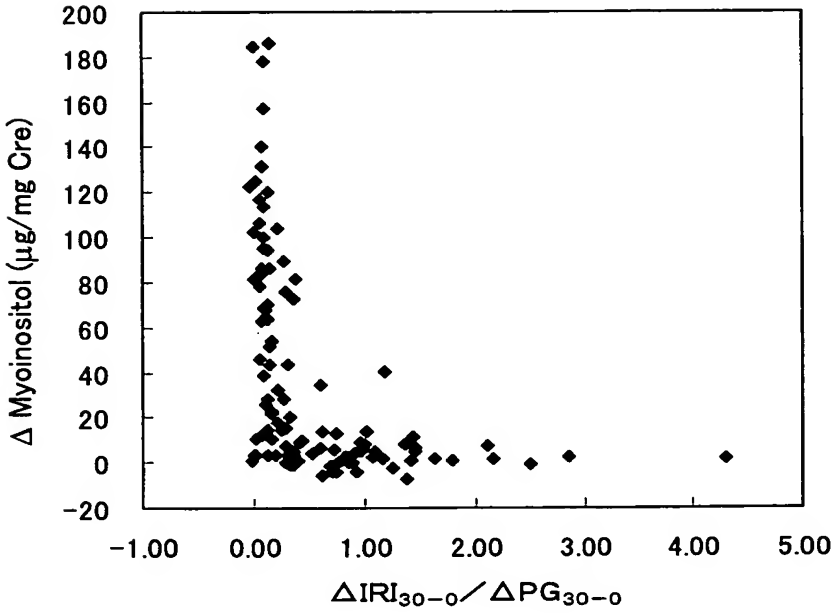

Δ Myoinositol (μg/mg Cre)
ΔIRI30-0／ΔPG30-0
200
180
160
140
120
100
80
60
40
20
0
-20
-1.00
0.00
1.00
2.00
3.00
4.00
5.00

Fig. 7

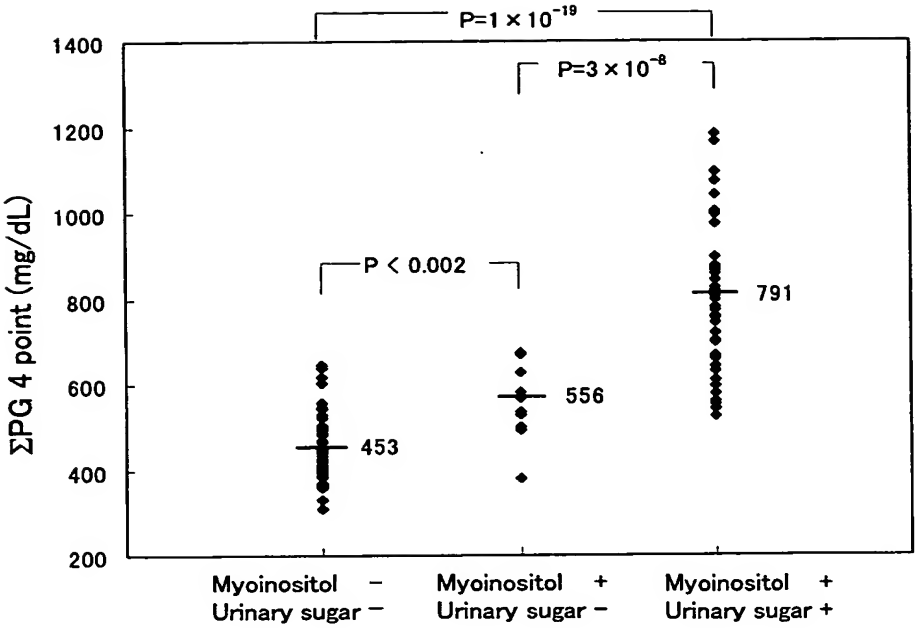

P=1 × 10^-19
P=3 × 10^-8
P < 0.002
ΣPG 4 point (mg/dL)
1400
1200
1000
800
600
400
200
453
556
791
Myoinositol −
Urinary sugar −
Myoinositol +
Urinary sugar −
Myoinositol +
Urinary sugar +

Fig. 8

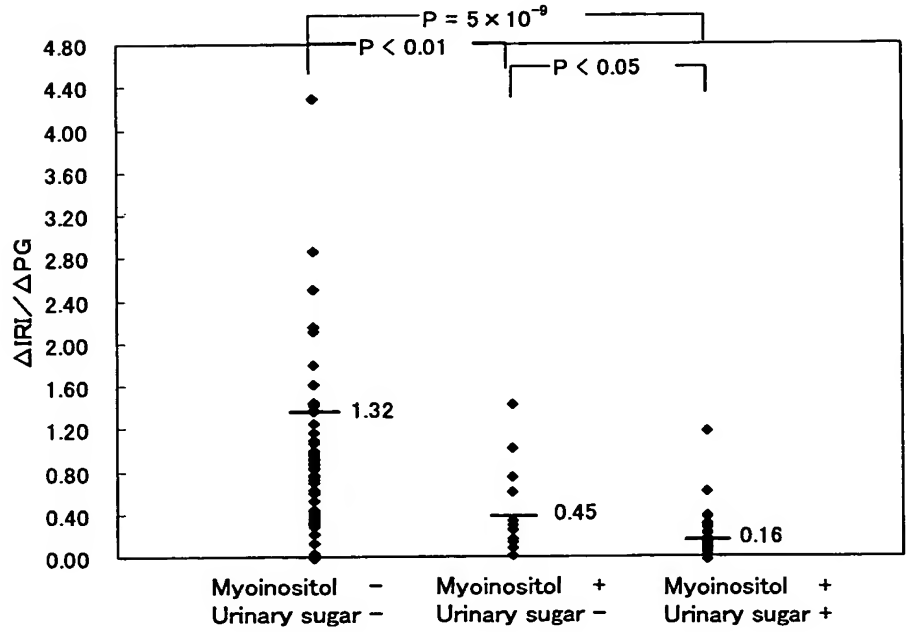

P = 5 × 10^-9
P < 0.01
P < 0.05
ΔIRI/ΔPG
4.80
4.40
4.00
3.60
3.20
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0.00
1.32
0.45
0.16
Myoinositol −
Urinary sugar −
Myoinositol +
Urinary sugar −
Myoinositol +
Urinary sugar +

Fig. 9

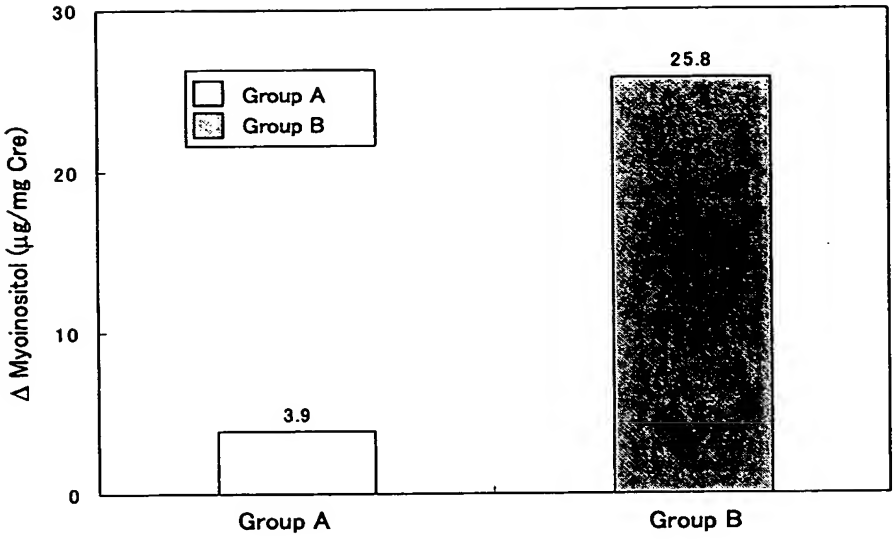
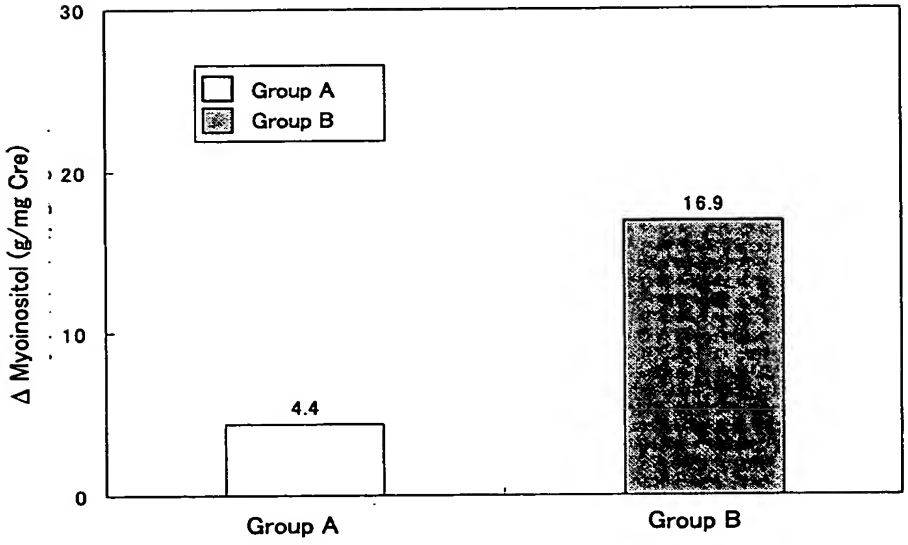

30
20
10
0
Δ Myoinositol (μg/mg Cre)
Group A
Group B
25.8
3.9
Group A
Group B

Fig. 10


30
20
10
0
Δ Myoinositol (g/mg Cre)
Group A
Group B
16.9
4.4
Group A
Group B